

Title: Decreased Clearance of CNS Amyloid- β in Alzheimer's Disease

Kwasi G. Mawuenyega¹, Wendy Sigurdson^{1,2}, Vitaliy Ovod¹, Ling Munsell¹, Tom Kasten¹, John C. Morris^{1,2,4}, Kevin E. Yarasheski⁵, Randall J. Bateman^{1,2,3*}

Departments of Neurology¹, Alzheimer's Disease Research Center², Hope Center for Neurological Disorders³, Pathology and Immunology⁴, Medicine⁵, Washington University School of Medicine, St. Louis, MO 63110, USA

* To whom correspondence should be addressed. E-mail: batemanr@wustl.edu

Materials and Methods

Research participants were enrolled from the Washington University Alzheimer's Disease Research Center. The inclusion criteria were age >60 years, cognitively normal (Clinical Dementia Rating or CDR 0), very mild AD (CDR 0.5) or mild AD (CDR 1). Exclusion criteria were other primary diagnosis of dementia or significant medical co-morbidity including stroke, bleeding diathesis, anticoagulation, and active infectious process. Full verbal and written informed consent was obtained from all participants. Twenty-four participants were clinically evaluated and rated as either very mild to mild dementia of the Alzheimer type (n=12, average age 77 +/- 7.3 years, range 61 to 85; Apolipoprotein E (ApoE) genotype 4/4 n=2, 3/4 n=4, 3/3 n=5, 2/3 n=1; gender 9 men, 3 women), or as cognitively normal (n=12 average age 70.6 years +/- 6.2, range 65 to 84; ApoE genotype 3/4 n=6, 3/3 n=5, 2/3 n=1; gender 4 men, 8 women). Following CSF sampling, three subjects had headaches and one required a blood patch. One participant discontinued the study because of pre-existing confusion at night.

Participants were admitted to the Washington University Clinical Research Unit at 7AM. Two intravenous and one lumbar intrathecal catheters were placed as previously described (Bateman et. al 2006). After baseline blood and CSF samples were collected, a primed bolus of $^{13}\text{C}_6$ -Leucine was infused at 2 mg/kg over 10 minutes, followed by 2 mg/kg/hr for the remaining 8 hours and 50 minutes. During and after infusion, CSF and blood samples were collected for a total of 36 hours and frozen at -70°C . Twelve hours after the lumbar catheter was removed, participants were discharged from the research unit.

All samples were processed and measured in a blinded fashion with data results and individual analysis completed before unblinding to participant's disease state. $\text{A}\beta_{42}$ and then $\text{A}\beta_{40}$ were serially immunoprecipitated from CSF samples using c-terminal specific antibodies, 21f12 and 2g3. The purified $\text{A}\beta$ was then digested with trypsin and $^{13}\text{C}_6$ -leucine abundance in these tryptic fragments quantified using tandem mass spectrometry as previously described (1, 2).

$^{13}\text{C}_6$ -leucine labeled media enrichment standards and CSF samples were analyzed using the Stable Isotope Labeling Tandem Mass Spectrometry (SILT MS) method (3) using SILTmass (4). Each tandem mass spectrometry scan was searched against the $\text{A}\beta$ FASTA sequence database to identify peptides and assign a score. SILTmass summed the signal intensity for tandem mass spectrometry ions that matched the theoretical *b* and *y* ions for $\text{A}\beta$ tryptic fragments and saved results as part of a pepXML file. A set of pepXML files for each subject was then analyzed to calculate the ratio of labeled $\text{A}\beta$ to unlabeled $\text{A}\beta$ by dividing the total labeled and unlabeled signal intensities. The percent $^{13}\text{C}_6$ -leucine labeled $\text{A}\beta$ was calculated as the ratio of $^{13}\text{C}_6$ -leucine- $\text{A}\beta_{17-28}$ divided by natural isotope abundance $\text{A}\beta_{17-28}$. Results were exported to Microsoft Excel, normalized by the slope of the standard curve and leucine labeling ratio ($^{13}\text{C}_6\text{-Leu}/^{12}\text{C}_6\text{-Leu}$) (5).

Calculation of the Fractional Synthesis Rate (FSR) and Fractional Clearance Rate (FCR) were performed as previously described (2) in a blinded fashion to dementia status. After calculations and measurements were made, the dementia status of each participant was used for statistical comparison. Comparison of FSR and FCR between cognitively normal and AD groups were made by two-tailed t-test with significance set at $p < 0.05$ (Graphpad 5.03).

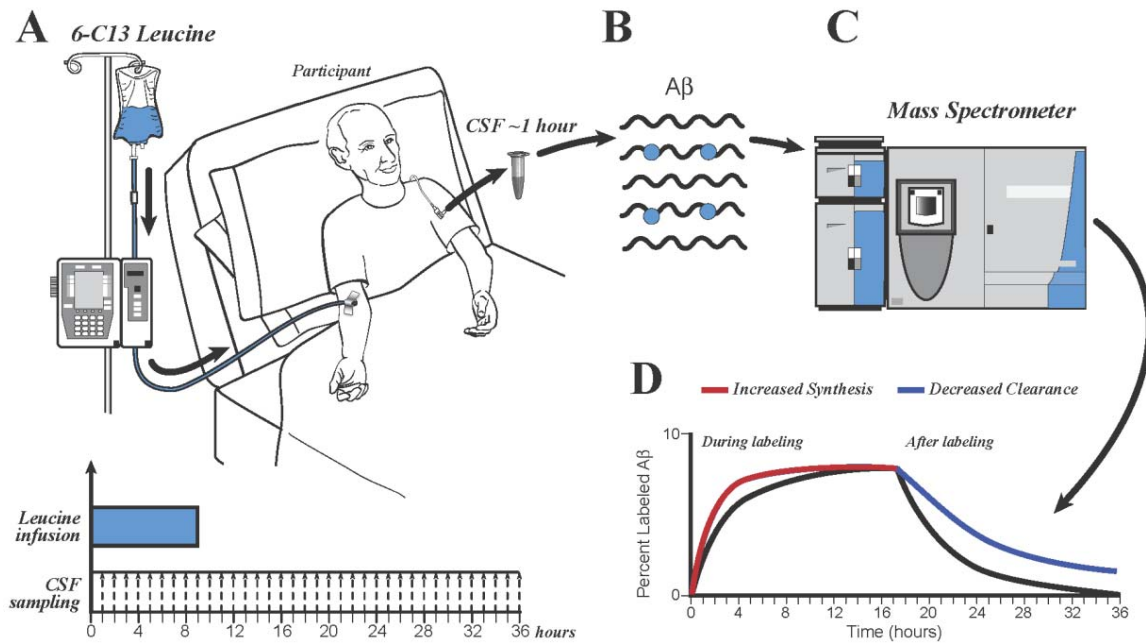


Fig. S1. Diagram of a stable isotope labeling kinetics study of A β production and clearance. (A) Participants are infused with a stable isotope labeled amino acid while cerebrospinal fluid samples are collected before, during, and after labeling. (B) A β is purified and processed for (C) mass spectrometry analysis of the amount of labeled and unlabeled A β present in the sample. (D) The increase in labeled A β during the production phase and the removal of labeled A β during the clearance phase reflects the relative production and clearance of A β in the CNS.

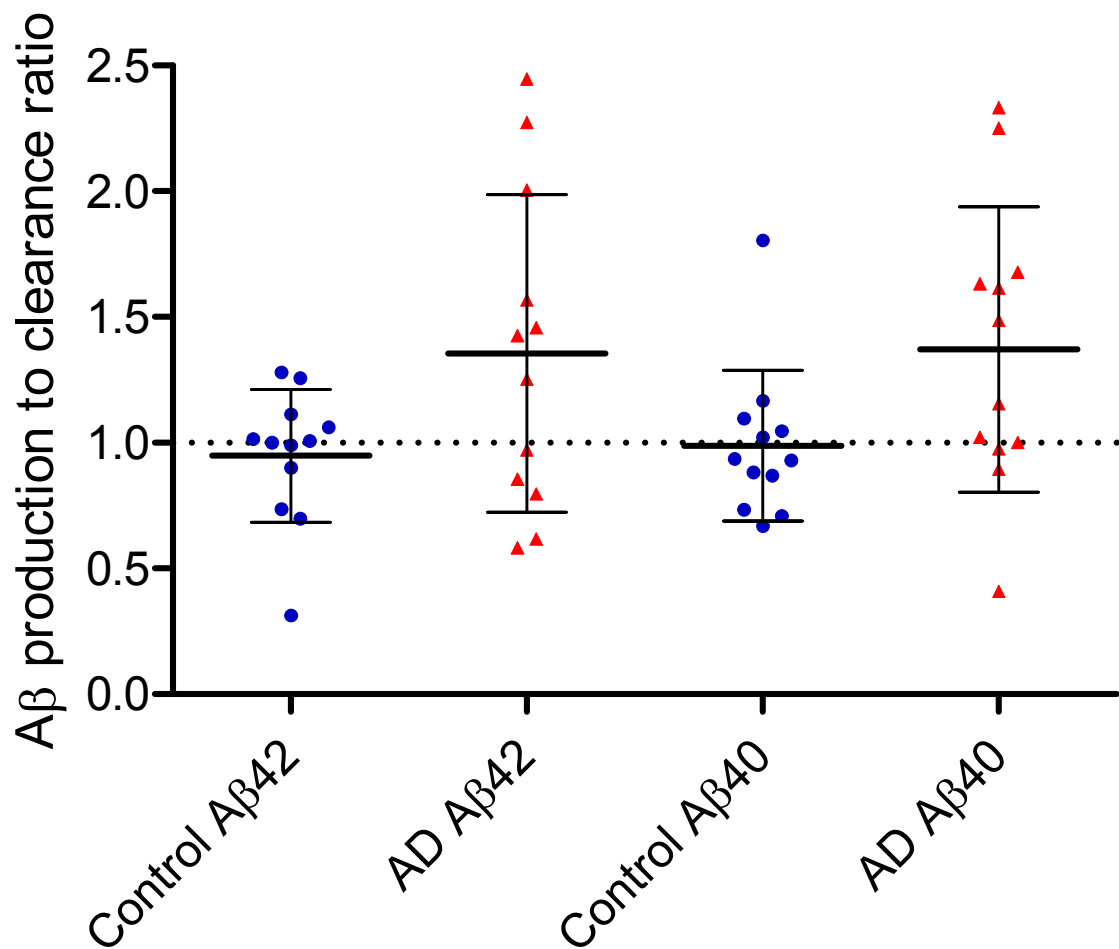


Fig. S2. Ratio of A β production to clearance in AD and controls. The ratio of A β 42 production to clearance rate is balanced in controls (0.95), while it is higher in those with AD (1.35). Similarly, there is increased A β 40 production to clearance ratios in AD (1.37) compared to controls (0.99). Means \pm standard deviations are plotted ($p < 0.05$ ANOVA)

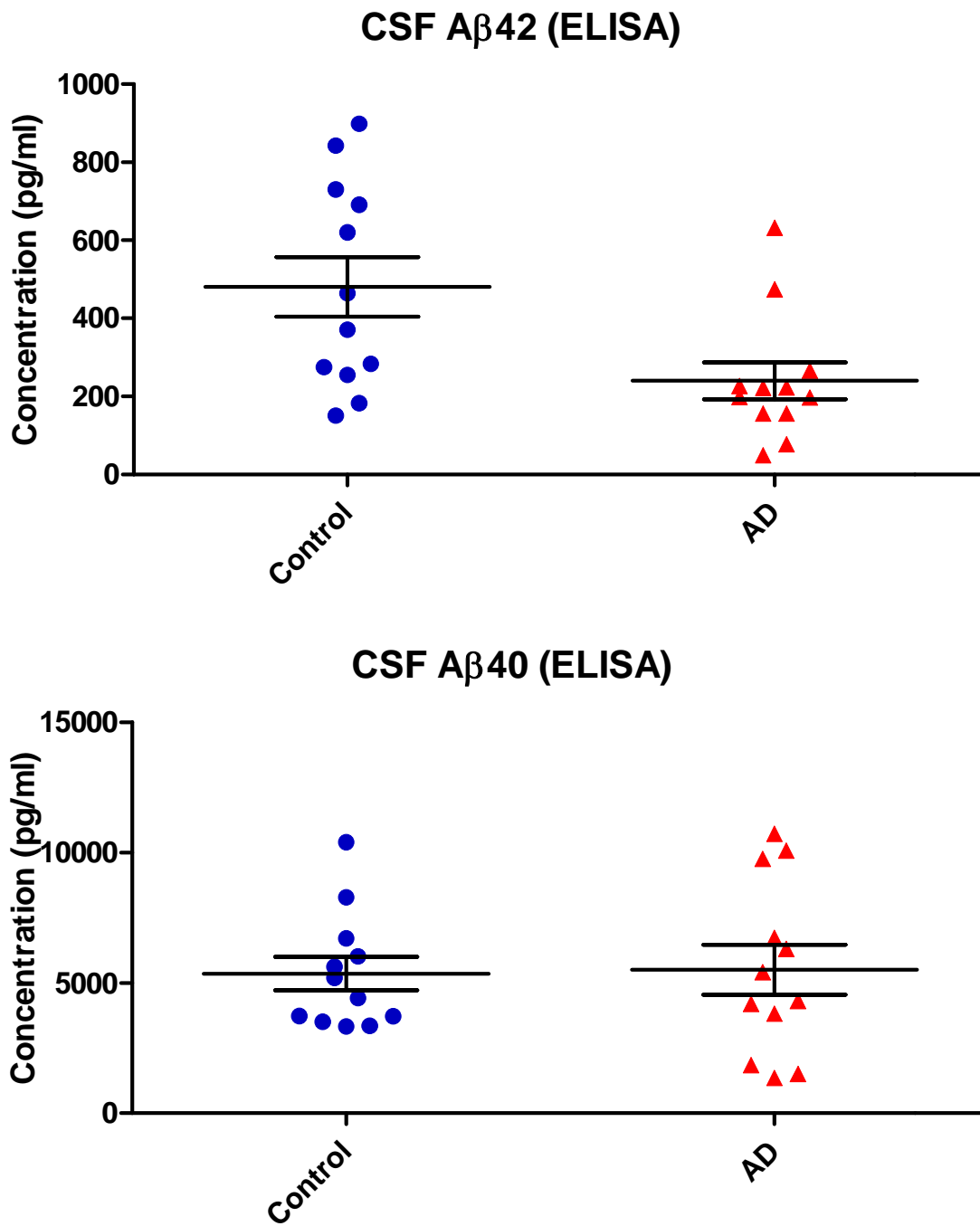


Fig. S3. Average CSF A β 42 and A β 40 concentrations. As expected CSF A β 42 concentrations were lower in the AD group compared to the control group ($p < 0.05$). Means \pm standard errors are plotted.

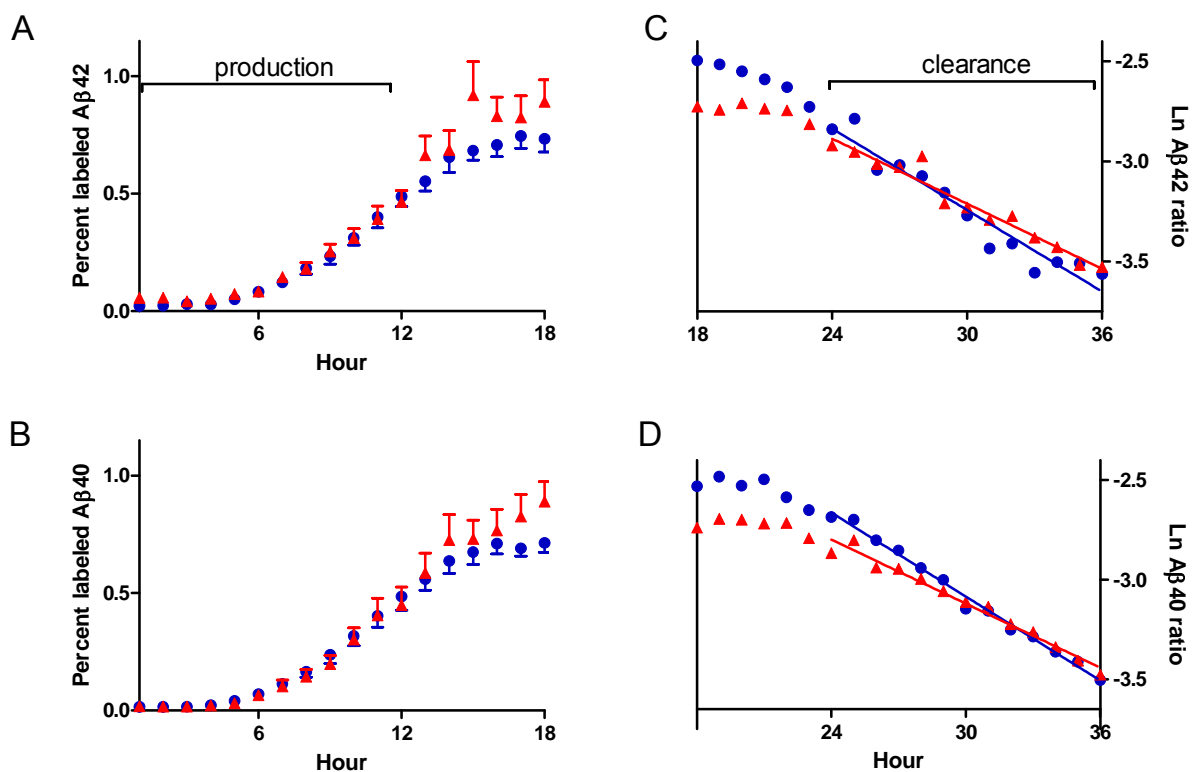


Figure S4: Complete time courses of the average Aβ kinetics in the CNS of twelve AD participants (red triangles) and twelve controls (blue circles). The average leucine normalized labeled Aβ42 (A) and Aβ40 (B) time course is shown for hours 1 to 18. The natural log (ln) plot of labeled to unlabeled ratio of Aβ42 (C) and Aβ40 (D) is shown for hours 18 to 36.

Supplemental References

1. R. J. Bateman *et al.*, *Ann Neurol*, (Mar 18, 2009).
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